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PROTECTION AGAINST IRON NEUROTOXICITY BY FACTORS RELEVANT IN MULTIPLE SCLEROSIS

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INTRODUCTION

Multiple sclerosis (MS) is the most common disabling neurological condition affecting young adults, and the prevalence of the disease in Canada, at 240 per 100000, is one of the highest in the world [1]. Neuronal degeneration is thought to contribute to progression in multiple sclerosis (MS). Iron accumulation in the central nervous system (CNS) of MS patients may lead to neuronal degeneration, partly by enhancing oxidative stress [2]. Women with MS tend to have fewer clinical relapses during pregnancy [3]. Minocycline, a tetracycline antibiotic with neuroprotective properties, has garnered interest in the treatment of MS [4]. Based on these observations, we hypothesized that pregnancy-associated hormones and minocycline would protect against iron neurotoxicity.

METHODS

Primary human foetal neuronal cultures were plated at 100,000 cell/well in 96-well plates and pretreated with ß-estradiol, progesterone, prolactin, allopregnanolone, and several tetracycline antibiotics for 24 hours (n=4). Cultures were then exposed to a 24-hour FeSO4 challenge, after which they were fixed with 4% paraformaldehyde. Immunocytochemistry was used to mark microtubule associated protein-2 (MAP-2), a neuronal marker in culture. MAP-2 positive neurons were quantified objectively using ImageXpress Micro.

RESULTS

24-hour exposure to FeSO4 resulted in significant, concentration-dependent loss of MAP-2 positive neurons. Pre-treatment with progesterone and minocycline preserved the number of MAP-2 positive neurons compared to FeSO4 alone. Protection by minocycline was dependent on its co-presence with iron, suggesting a chemical interaction that may include alleviating oxidative stress; removing minocycline prior to iron exposure negated the protection. The effect of minocycline was compared to that of four other tetracycline antibiotics (tetracycline, oxycycline, doxycycline, demeclocycline), and only minocycline resulted in a preservation of MAP-2 positive neurons compared to iron alone. At the concentrations investigated, none of the pregnancy hormones examined (i.e. progesterone, ß-estradiol, estriol and allopregnanolone at 300ng/mL and prolactin at 30nM) were protective against iron.

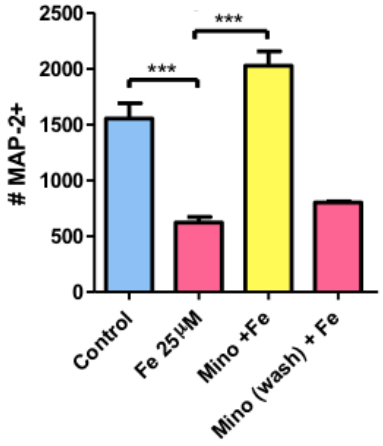

Figure 1. Mean number of MAP-2 positive neurons present in each treatment group (+SEM; n=4). Minocycline concentration is 10µg/mL, and Fe^{2+} is a 25µM $FeSO_4$ challenge. Both minocycline pre-treatment and Fe^{2+} challenge times were 24 hours. In the "wash" group, contents of the wells were removed before addition of the next treatment. Data were analyzed using a one-way ANOVA followed by the Bonferroni post-hoc test. ***p>0.001.

DISCUSSION AND CONCLUSIONS

The accumulation of iron in the CNS of MS can have neurotoxic outcomes. This results of this experiment showed that minocycline can protect against the neurotoxicity of iron, possibly through a mechanism involving chemical interaction that could include countering oxidative stress. Since minocycline is being investigated in the treatment of MS [4], these results may bode well for its therapeutic efficacy. Furthermore, these findings may aid in development of treatments to reduce neurodegeneration in MS and other conditions involving iron accumulation and neurotoxicity.

REFERENCES

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